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Environmental sources of rapid growing nontuberculous mycobacteria causing disease in humans

J. van Ingen[1,2], M. J. Boeree[1], P. N. R. Dekhuijzen[1] and D. van Soolingen[2]

1) *Department of Pulmonary Diseases, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands and* 2) *National Mycobacteria Reference Laboratory, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands*

Abstract

Nontuberculous mycobacteria are environmental, opportunistic pathogens whose role in human disease is increasingly recognized, especially regarding the rapid growing mycobacteria (RGM). RGM are recovered from various environmental sources, both natural and man-made. In water systems, RGM can survive by forming biofilms and by interactions with protozoa. The presence and species diversity of RGM in water is influenced by temperature, pH and the chemical quality of the water, as well as the availability of nutrients, although the exact correlations remain controversial. Despite their omnipresence in environmental sources, the actual transmission of RGM to humans, with subsequent clinical disease, has rarely been proven. However, outbreaks as a result of contaminated water sources have been reported, although accidental presence in clinical samples cannot always be excluded. In this setting, the presence of RGM does not necessarily indicate a causal relationship with clinical disease; accidental presence in clinical samples cannot always be excluded. Future studies should focus on the exact environmental sources of infection, aiming to examine possibilities for prevention of infections in patients at risk. Furthermore, studies should focus on the actual sites of the active replication of RGM; their presence may not indicate their natural habitat.

Keywords: Atypical, aetiology, *Mycobacterium* infections, rapid growers, review

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Corresponding author and reprint requests: J. van Ingen, National Institute for Public Health and the Environment (RIVM; LIS), Mycobacteria Reference Laboratory, Po Box 1, 3720 BA Bilthoven, The Netherlands
E-mail: jakko.van.ingen@rivm.nl

Introduction

Nontuberculous mycobacteria (NTM) are environmental, opportunistic pathogens that are increasingly recognized as causative agents of human disease [1]. Among NTM, the rapid growing species have recently gained increasing attention because they are associated with specific disease types in specific patient categories, and are characterized by extensive resistance to antimicrobial drugs [1]. The term 'rapidly growing mycobacteria' was defined by Runyon [2,3] to include mycobacteria that form mature colonies on solid agar in 7 days from subculture. The first documented clinical NTM isolates, in the early 20th century, were all rapid growing mycobacteria (RGM), which, at the time, were often considered to be members of *Nocardia* or other genera [4].

Presently, in the Netherlands, RGM as a group comprise 13% of all NTM isolates submitted to the national mycobacteria reference laboratory. Bacteria of the *Mycobacterium chelonae–Mycobacterium abscessus* group are the most frequently encountered species, comprising 45% of all referred RGM; the *Mycobacterium fortuitum* complex members comprise another 35% (National Mycobacteria Reference Laboratory).

The environment is the suspected source of human RGM disease [1], although not all species have been isolated from environmental sources. In previous environmental studies, RGM comprised a small minority of the NTM isolates, ranging from 2% in a North American study of drinking water distribution systems (DWDS) [5] to 35% in a Dutch survey of tap, swimming pool and whirlpool water [6].

In this review, we summarize the currently available data on isolation of RGM from environmental sources, the human disease that is directly attributable to exposure to these sources, as well as the techniques available for investigating transmission from environmental sources to humans.

Concerning the review criteria, relevant English-language publications were identified using the PubMed database. The Medical Subject Heading term '*Mycobacterium* infections, atypical/aetiology' was used.

The Presence of RGM in Natural Environments

Soon after the discovery of *M. tuberculosis* by Robert Koch, the first reports on mycobacteria in environmental samples appeared [4]. Subsequently, NTM have been isolated from environmental samples, mostly water and soil, worldwide. Most reports, however, have focused on the presence of slow growing NTM, especially *Mycobacterium avium* complex (MAC) members, in water samples [5,7].

Water

The presence of RGM in natural water sources of widely varying composition has been recorded in many countries. Relatively few attempts have been made to recover RGM from salt water. The first published report stems from Norway, where various unidentified RGM were cultured from water samples from the Bergen city harbour [8]. Gruft *et al.* [9,10] subsequently sampled coastal waters in the USA. From these samples, rapid growers identified as '*M. fortuitum–Mycobacterium chelonei* complex' were isolated, albeit in <1% of the specimens [10]. Overall, the number of NTM isolates from their water samples dwindled if the salinity exceeded 2% [10]. An interesting recent contribution to this field was the case series of RGM (mainly *M. abscessus* and *M. fortuitum*) skin infections observed after the 2004 tsunami in south-east Asia [11]. These infections most likely resulted from contact with sea water, although NTM may have entered the water from soil or plants.

Data on NTM isolation from fresh water are more extensive, although few reports focus on RGM and fewer have identified these. The first detailed reports on RGM isolation from fresh water stem from South Wales, where Paull *et al.* [12,13] isolated various unidentified RGM from lake and river samples. Reports from other countries followed. In Finland, various unidentified RGM were cultured from brook waters [14]. In the USA, surface waters yielded *Mycobacterium mucogenicum*, *M. chelonae* and *M. fortuitum* complex isolates [15]. A large study on the NTM ecology of the Rio Grande River revealed that *M. fortuitum* complex members were most frequently isolated, though *Mycobacteriem mageritense* and *Mycobacteriem phlei* were also recovered [16]. Our own group recently cultured an *M. fortuitum* complex organism from ground water of a village water pump in Northern Afghanistan (J. van Ingen; personal communication). *Mycobacterium conceptionense*, a novel *M. fortuitum* complex member, was cultured from a patient with osteitis of an open fracture after prolonged immersion in a river on Reunion Island near Madagascar [17]. Thus, RGM are present in natural waters worldwide, representing a potential source of human disease.

Soil

Considering the number of available reports, soil sampling is less popular then water sampling, possibly as a result of technical difficulties with contamination of cultures. The early experiments by Beerweerth, reviewed by Collins *et al.* [13], revealed that most NTM species can grow in arable or pasture land samples; forest soils, peat and leaf mould supported growth of few NTM species. In subsequent decades, various RGM species have been isolated from diverse soil types in many different countries. In south India, *M. fortuitum* complex organisms were frequently isolated, along with other RGM rarely involved in human disease, including *M. phlei*, *Mycobacterium gadium* and *Mycobacterium diernhoferi* [18]. In studies in Malawi, a similar predominace of *M. fortuitum* complex members was noted [19]. In wheat plantation soils in the UK, various unidentifiable RGM related to *Mycobacterium austroafricanum* were found [20].

De Groote *et al.* were the first to assess the presence of NTM in potting soils of patients with pulmonary NTM disease and found *M. chelonae* in potting soils of a patient with MAC and *M. chelonae* disease [21].

In the soil, some RGM species perform an interesting function, namely the clean-up of pollution by polycyclic aromatic hydrocarbons and other pollutants. Polycyclic aromatic hydrocarbons comprise a class of mutagenic and carcinogenic organic pollutants produced by fossil fuel combustion, human industrial activity, and also volcanic activity [22].

Presence of RGM in Man-made Environments

Most studies on environmental sources of NTM infection have focused on the man-made environments that we are all exposed to on a daily basis (e.g. tap water). All NTM are common inhabitants of DWDS, and thus tap water. The proportion of RGM among NTM in DWDS differed among studies. In an extensive survey in the Netherlands, 30–40% of all NTM detected in tap, swimming pool and whirlpool water samples were RGM. Unfortunately, very few were identified

to the species level [6]. In Finland, investigators recorded that <7% of all NTM cultured from DWDS were RGM (although these were not further identified) [23]. In Paris (France), RGM comprised almost 50% of all identifiable NTM in the DWDS; *M. chelonae*, *M. fortuitum*, *Mycobacterium peregrinum* and *M. gadium* were recovered [24]. More recently, cooling towers in Paris yielded *M. chelonae*, *M. fortuitum*, *M. conceptionense* and *Mycobacterium phocaicum* [25]. In other regions, different species distributions were noted. In South Africa, *Mycobacterium gilvum*, *M. abscessus* and *M. fortuitum* complex members were isolated from DWDS [26]. Studies conducted in the USA revealed important differences in the distribution of NTM in tap water derived from surface water sources (where rapid growing *M. mucogenicum* and *M. chelonae* dominated) and from ground water (where the slow growing *M. gordonae* dominated) [15,27]. Total mycobacterial counts within a DWDS are highest in the most distal sites of the system, mainly in deposits. This suggests that the NTM can actually grow in the systems, although this has not been proven [5,23].

Biofilms

Biofilms are microbial communities that attach to a surface interface and to each other, producing an extracellular matrix of polymeric substances for their maintenance. Within a biofilm, bacteria can switch to a different phenotype, growth rate and gene transcription profile [28]. Biofilms are important sources of RGM and may be elementary to their survival in DWDS. Their hydrophobicity and metal resistance make NTM biofilm pioneers [29]; the RGM *M. fortuitum* and *M. chelonae* have been shown to form biofilms even in permanently running water [30]. Embedded in a biofilm, RGM are more protected against antimicrobial agents than in a planktonic state [28].

Metal-working fluid

The RGM *Mycobacterium immunogenum* merits special attention because it has traditionally been associated with a peculiar man-made environment, metal-working fluid. It was first described as the causative agent of an outbreak of hypersensitivity pneumonitis among factory workers, who sprayed a semisynthetic metal-working fluid on their machines to cool them [31].

Interactions with protozoa

RGM can interact with the free-living amoebae and other protozoa present in water systems [32]. Inside amoebae, the mycobacteria are further protected from hostile environments. Association with amoebae may even select for organisms with increased capability of infecting humans; mechanisms needed for survival within amoebae may be similar to those needed within human macrophages, as observed for *Legionella* spp. [33]. Recently, these protozoal interactions have been exploited, by performing amoebal co-culture, to isolate RGM from environmental samples [25].

Determinants of the Environmental Presence of RGM

Whereas RGM are commonly found in environmental samples, determinants for their survival and growth remain largely unknown. The water or soil composition is likely to exert a strong effect on the presence of NTM and species distribution. The presence of NTM has been positively associated with water and soil acidity [7,14], organic matter (including humic and fulvic acids), zinc and iron content, and total bacterial counts [6,14,23,34]. By contrast, previous studies reported negative associations between the presence of NTM and the chemical toxicity of water [16], especially the free chlorine content [5,6]. Nevertheless, most of these associations remain the subject of debate.

Despite positive associations between the acidity of both water and soil samples and the presence of NTM [7,14,34], the presence of NTM was also noted in the relatively alkaline waters of the Rio Grande river in the southern USA [16]. Similarly, in soil samples in Malawi, no effect of pH on NTM counts was observed [19]. Similarly, not all studies found an effect of chlorination [13,23]; the RGM *M. chelonae* and *M. fortuitum* are especially resistant to chlorination [24].

The effect of temperature on the presence of NTM is equally controversial; the presence of RGM has been associated with high [6,7] as well as low water or soil temperatures [16,18]; other studies noted no effect of temperature [24]. The heat susceptibility of NTM differs by species, including among different RGM [35].

The final controversy is the association between NTM counts and total coliforms in water samples. Some studies have recorded positive correlations between the amount of NTM in natural environments and the levels of coliforms and suggest that they favour similar conditions for replication and persistence, or a similar source from which they are introduced into water (e.g. animal fecal material) [16]. The general consensus, however, is that no such correlations exist [6,26,29,36].

Important differences in the geographic origin, pH, temperature and mineral composition of the water samples from the various studies hamper the interpretation of the effect

of single variables on RGM presence. Many of the variables presented in this review may exert a selective pressure rather than influence total mycobacterial counts in the environment [29]. Moreover, these variables may have less impact on RGM living in biofilms or protozoa.

Limitations of Environmental Studies

Even though RGM have been detected in a wide range of environmental samples, their actual natural habitat remains uncertain. The debate concerning whether they are principally inhabitants of soil or water has been reviewed previously [13], and still continues. Considering the large number of NTM species and the considerable differences in species distribution in water and soil [7,18,19,37], niches may differ among NTM species [5]. Moreover, their presence in water or soil samples may not indicate replication and persistence in this environent *per se*. For the important human pathogen *Mycobacterium ulcerans*, which is not a RGM, experiments have been conducted to determine the actual sites of replication of these bacteria. Crude extracts from two aquatic plants added to culture medium halved the doubling time of *M. ulcerans* and promoted biofilm formation [38]. Similar research is warranted for RGM, to elucidate the sites of active replication. To date, studies have mainly looked into associations of RGM presence with environmental characteristics.

Many studies are limited by their detection and identification methods. The use of cultures represents an important bottleneck; fastidious species are easily missed. Moreover, decontamination procedures, selection and enrichment of media, incubation temperatures, as well as the duration of incubation, may all affect the yield of cultures and introduce important selection bias [27]. The use of direct PCR and cloning is technically more demanding, although it may offer a more complete scope on the presence of NTM in environmental samples [19,20]. For RGM, the *rpoB* gene may be the best target; sequencing of this gene has provided important contributions to our understanding of RGM taxonomy [25]. Historically, many studies have focused on specific species, generally MAC, applying identification tools geared towards these species [5,7,9,10,37]; these underestimate NTM species diversity in environmental samples.

The other important issue is the high number of unidentifiable species in environmental samples. Using molecular methods, many studies have reported high percentages of unidentifiable NTM, ranging from 36% (n = 126/351) [23] to 55% (n = 57/104) [24]. Many of these NTM may represent new species; whether it is meaningful to describe them as such remains a subject of debate [39].

Transmission to Humans

RGM are present in wide ranges of natural and man-made environments and, from here, transmission to humans occurs. The presence of NTM species in environmental samples of specific regions is associated with increased sensitization to these species, as observed in skin testing [37], although this has not yet been extended to the RGM.

Transmission to humans is important for two reasons. First, the environmental RGM can cause clinical disease in humans after exposure [1]. Second, the exposure to environmental RGM may alter the efficacy of bacillus Calmette-Guérin vaccination in humans and thus influence the struggle to curb the tuberculosis epidemic [18,19].

In part because of difficulties related to timely and comprehensive sampling, very few studies have been able to relate clinical RGM disease to exposure from natural environments [21]. In RGM outbreaks associated with contaminated man-made (often hospital) environments, bacterial typing studies are more successful in determining the source of transmission to humans [40–42].

Tracking transmission: molecular typing techniques

Various typing techniques can be used to type RGM and determine the possible sources of human infections. For a long time, pulsed-field gel electrophoresis has been the reference standard typing method, despite problems of DNA degradation in *M. abscessus* isolates. Other generic methods in use are random amplified polymorphic DNA typing [43] and repetitive sequence-PCR, which has recently become available in an automated system (DiversiLab™; BioMerieux, Marcy l'Etoile, France) [44]. For *M. abscessus*, the random amplified polymorphic DNA technique has the advantage in that the methodology and interpretation have been formally described [43]. Typing methods targeting species-specific insertion sequences, such as IS*1245* in *M. avium* [45], do not yet exist for RGM.

Conclusions

In summary, the cosmopolitan RGM are recovered from various environments, both natural and man-made, around the globe. In most environmental studies, RGM comprised a minority of the NTM isolates. Probably as a result, these RGM generally remained unidentified and were not, as a separate group, related to chemical or biological characteristics of the environmental sample from which they were derived.

The potentially pathogenic *M. fortuitum* complex isolates are especially frequent in soil samples but are also present in water; the equally pathogenic *M. chelonae–M. abscessus* group organisms are mostly recovered from water samples and infrequently from soil. For RGM, actual transmission to humans from an environmental source, with subsequent clinical disease, is rarely proven. Exceptions to this rule are outbreaks as a result contaminated (hospital) water sources. Future studies should focus on the exact environmental sources of infection, to examine possibilities for the prevention of infections in patients at risk. Furthermore, studies should focus on the actual sites of the active replication of RGM; their presence may not indicate replication and persistence *per se*.

Transparency Declaration

None of the authors have any relationship with any entity (commercial or non-commercial) that has an interest in the subject matter of this review.

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